

What is claimed is:

1. A method of screening for compounds that inhibit melanogenesis, the method comprising: treating cells expressing a tyrosinase-encoding gene with a test compound, and determining the cellular localization of tyrosinase in the presence of the test compound; wherein a change in the cellular localization of tyrosinase in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that inhibits melanogenesis.
2. The method of claim 1, wherein the cells further express a P protein-encoding gene, and wherein the change in the cellular localization of tyrosinase in the presence of the test compound as compared to in the absence of the test compound is dependent upon the expression of the P protein-encoding gene.
3. The method of claim 1 or 2, wherein the cellular localization of tyrosinase is determined by assaying the amount of tyrosinase secreted by the cells in the presence of the compound, wherein an increase in the amount of tyrosinase secreted by the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that inhibits melanogenesis.
4. The method of claim 1 or 2, wherein the cellular localization of tyrosinase is detected by assaying for tyrosinase activity.
5. The method of claim 1 or 2, wherein the cellular localization of tyrosinase is detected by assaying for the presence of tyrosinase protein using immunological techniques.
6. The method of claim 1 or 2 further comprising the step of assaying the amount of tyrosinase associated in a high molecular weight complex in the presence of the test compound, wherein a decrease in the amount of tyrosinase associated in a high molecular weight complex in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that inhibits melanogenesis.
7. The method of claim 1 or 2 further comprising the step of assaying the amount of TRP-1 or TRP-2 protein associated in a high molecular weight complex in the presence of the compound, wherein a decrease in the amount of TRP-1 or TRP-2 protein associated in a high molecular weight complex in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that inhibits melanogenesis.

8. The method of claim 1 or 2 further comprising the step of assaying the number or size of melanosomes in the cells in the presence of the compound, wherein a decrease in the number or size of melanosomes in the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound inhibits melanogenesis.
- 5
9. The method of claim 1 or 2 further comprising the step of assaying the mass or length of tyrosinase in the cells in the presence of the compound, wherein a decrease in the mass or length of tyrosinase in the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound
- 10
- 15
10. The method of claim 1 or 2 further comprising the step of assaying for the levels and/or targeting of lysosomal hydrolases in the cells in the presence of the compound, wherein a decrease in accumulation of lysosomal hydrolases that are transported via the M6P/IGF-II receptor in the lysosome in the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that inhibits melanogenesis.
11. The method of claim 1 or 2, wherein the cells are grown in the presence of low tyrosine.
12. The method of claim 11 wherein the concentration of tyrosine is 0.01-0.03 mM.
- 20
13. The method of claim 1 wherein the cells are melanocytes.
14. The method of claim 1 wherein the cells are melanoma cells.
15. The method of claim 1 wherein the cells are derived from a mammal.
16. The method of claim 15 wherein the mammal is a human.
17. The method of claim 15 wherein the mammal is selected from the group consisting of
- 25
- mouse, hamster, and guinea pig.
18. A method of screening for compounds that increase melanogenesis comprising: treating cells expressing a tyrosinase-encoding gene with a test compound, and determining the amount of tyrosinase secreted by the cells in the presence of the test compound; wherein a decrease in the amount of tyrosinase secreted by the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that increases melanogenesis.
- 30

19. The method of claim 18 wherein the cells further express a P protein-encoding gene, and wherein the decrease in the amount of tyrosinase secreted by the cells in the presence of the test compound as compared to in the absence of the test compound is dependent upon the expression of the P protein-encoding gene.
- 5 20. The method of claim 18 or 19 further comprising determining a ratio of tyrosinase inside the cells to tyrosinase secreted by the cells, wherein an increase in the ratio in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound induces melanogenesis.
- 10 21. The method of claim 18 or 19, wherein the amount of tyrosinase is detected by assaying for tyrosinase activity.
22. The method of claim 18 or 19, wherein the amount of tyrosinase is detected by assaying for the presence of tyrosinase protein using immunological techniques.
23. The method of claim 18 wherein the cells are melanocytes.
24. The method of claim 18 wherein the cells are melanoma cells.
- 15 25. The method of claim 23 or 24, wherein the cells are visually examined for an increase in melanin production.
26. The method of claim 23 or 24 wherein the cells do not express P protein, and wherein a decrease in the amount of tyrosinase secreted by the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound is a candidate for a compound that mimics P protein function.
- 20 27. The method of claim 23 wherein the cells are mouse melan-p melanocytes.
28. The method of claim 18 wherein the cells are derived from a mammal.
29. The method of claim 28 wherein the mammal is a human.
30. The method of claim 28 wherein the mammal is selected from the group consisting of mouse, hamster, and guinea pig.
- 25 31. The method of claim 26, wherein the cells are visually examined for an increase in melanin production.
32. A method of screening for compounds that affect the function of P protein, the method comprising: contacting a system with a test compound, the system comprising P protein and tyrosinase; and identifying those test compounds that affect tyrosinase activity in the system in a P protein-dependent manner.

33. The method of claim 32 wherein the system is a cell that expresses a P protein-encoding gene and a tyrosinase-encoding gene.
34. The method of claim 33 wherein the cell is a cultured cell.
35. The method of claim 32 wherein compounds that decrease tyrosinase activity in the system are identified as compounds that inhibit the function of P protein.
36. The method of claim 35 further comprising the step of assaying for the targeting of lysosomal hydrolases in the cells in the presence of the compound, wherein a decrease in accumulation of lysosomal hydrolases that are transported via the M6P/IGF-II receptor in the lysosome in the cells in the presence of the test compound as compared to in the absence of the test compound indicates that the test compound inhibits the function of P protein.
37. The method of claim 32 wherein compounds that result in an increase in tyrosinase activity in the system are identified as compounds that increase the function of P protein.
38. The method of claim 32 wherein the P protein-encoding gene is derived from a mammal selected from the group consisting of human, hamster, guinea pig, and mouse.
39. The method of claim 32 wherein the tyrosinase-encoding gene is derived from a mammal selected from the group consisting of human, hamster, guinea pig, and mouse.
40. A method of screening for compounds that affect the function of P protein, the method comprising: using a primary screening method to preliminarily determine whether a test compound may affect P protein function; and using one or more secondary screening methods to determine whether the test compound affects P protein function.
41. The method of claim 40, wherein the primary screening method comprises at least one screening assay selected from the group consisting of assaying for secretion of tyrosinase and assaying for the missorting of at least one lysosomal hydrolase.
42. A method of screening for compounds that affect the function of P protein, the method comprising: modeling a compound that affects the function of the P protein; making chemical analogs of the compound; and assaying the chemical analogs for their effect on the function of P protein.
43. The method of claim 42 wherein the compound is imipramine.
44. A method of decreasing melanin production in a melanocyte, comprising contacting the melanocyte with an effective amount of a compound that effects an alteration in late endosomal/lysosomal trafficking in the melanocyte, wherein the alteration in late endosomal/lysosomal trafficking results in a decrease in melanin production in the melanocyte.

45. The method of claim 44, wherein the alteration in late endosomal/lysosomal trafficking is effected by contacting the melanocyte with a compound that is an antagonist of late endosomal/lysosomal trafficking.
46. The method of claim 44, wherein the alteration in late endosomal/lysosomal trafficking is an alteration in late endosomal/lysosomal cholesterol trafficking.
- 5 47. The method of claim 44, wherein the alteration in late endosomal/lysosomal trafficking is effected by contacting the melanocyte with a compound selected from the group consisting of
- 10 (a) progesterone,
- (b) a hydrophobic amine,
- (c) sphingosine, and
- 15 (d) a compound of the formula
- wherein X is O or S;
- 15 R₁ is $-\text{C}(\text{O})(\text{C}_1\text{-C}_6)\text{alkyl}$ or $-(\text{CH}_2)_n\text{-O-}(\text{C}_1\text{-C}_6)\text{alkyl}$, or $-(\text{CH}_2)_n\text{-NR}_7\text{R}_8$ where n is 0-3, and where each of R₇ and R₈ are independently selected from H and (C₁-C₆)alkyl;
- 20 R₂ is H or (C₁-C₆)alkyl;
- R₃ is H or (C₁-C₆)alkyl;
- R₄ is $-\text{C}(\text{O})(\text{C}_1\text{-C}_6)\text{alkyl}$;
- 25 R₅ is H or $-(\text{C}_1\text{-C}_6)\text{alkyl}$; or R₄ and R₅ together are =O; and
- R₆ is H or $-(\text{C}_1\text{-C}_6)\text{alkyl}$ or $-(\text{CH}_2)_n\text{-NR}_9\text{R}_{10}$ where each of R₉ and R₁₀ are independently selected from H and (C₁-C₆)alkyl.
48. The method of claim 47, wherein the compound is progesterone.
49. The method of claim 47, wherein the compound is a hydrophobic amine.
- 25 50. The method of claim 49, wherein the hydrophobic amine is selected from the group consisting of a phenothiazine, and a tricyclic antidepressant.

51. The method of claim 50, wherein the compound is a phenothiazine.

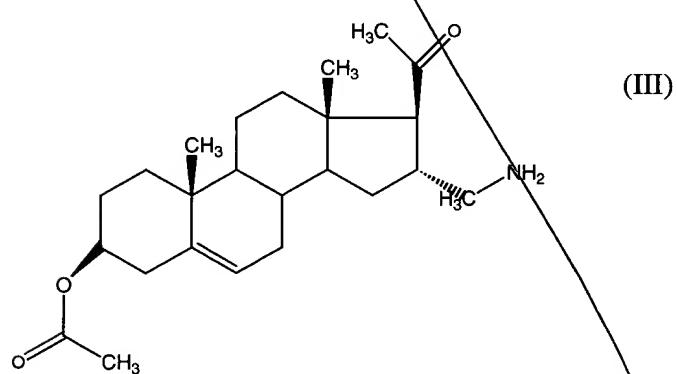
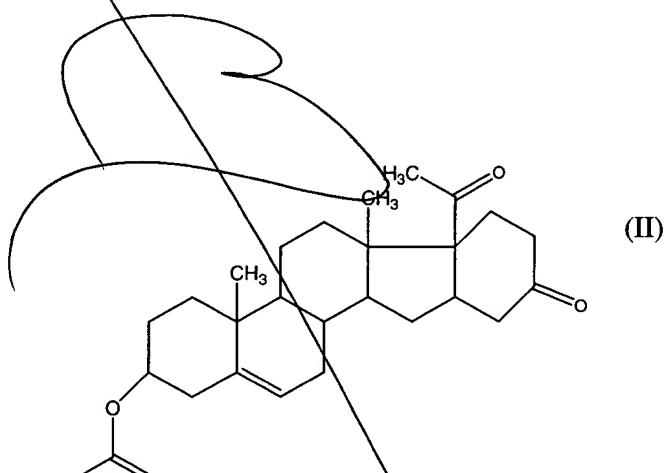
52. The method of claim 51, wherein the phenothiazine is selected from the group consisting of trifluoperazine, chlorpromazine, prochlorperazine, triflupromazine, promazine, thioridazine, mesoridazine, piperacetazine, perphenazine, fluphenazine, acetophenazine, and thiethylperazine.

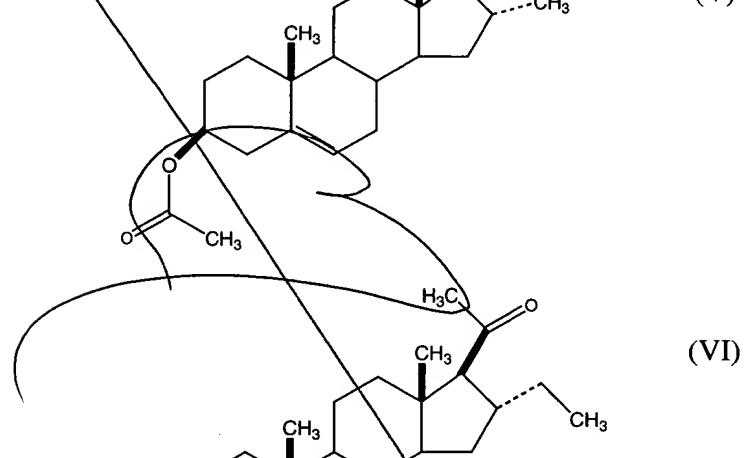
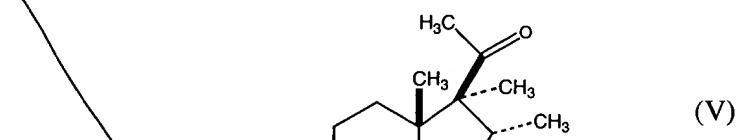
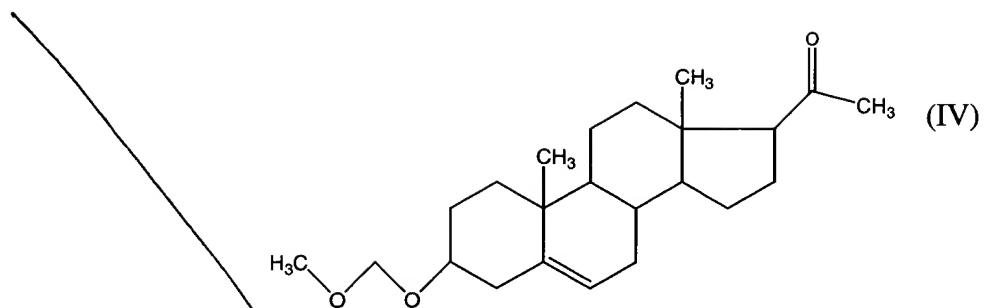
53. The method of claim 50, wherein the compound is a tricyclic antidepressant.

54. The method of claim 53, wherein the tricyclic antidepressant is selected from the group consisting of imipramine, nortriptyline, protriptyline, trimipramine, and doxepin.

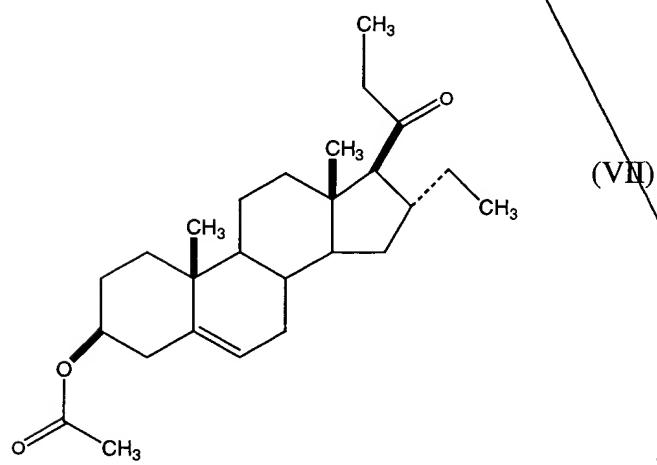
55. The method of claim 47, wherein the compound is sphingosine.

10 56. The method of claim 47, wherein the compound is selected from the group consisting of:

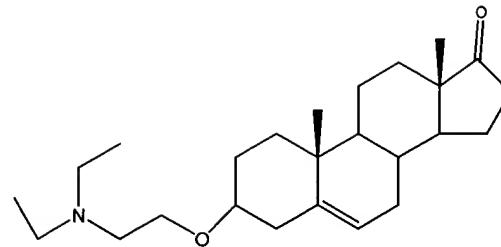




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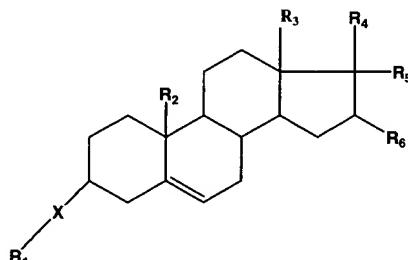
and



(VIII)

or a pharmaceutically acceptable salt or solvate thereof.

57. A method of reducing skin pigmentation, comprising contacting skin with a pharmaceutically effective amount of a compound that effects an alteration in late endosomal/lysosomal trafficking, wherein an alteration in late endosomal/lysosomal trafficking results in a reduction of skin pigmentation.
58. The method of claim 57, wherein an alteration in late endosomal/lysosomal trafficking is effected by contacting the skin with a compound that is an antagonist of late endosomal/lysosomal trafficking.
59. The method of claim 57, wherein the alteration in late endosomal/lysosomal trafficking is an alteration in late endosomal/lysosomal cholesterol trafficking.
60. The method of claim 57, wherein the alteration in late endosomal/lysosomal trafficking is effected by contacting the skin with a pharmaceutically effective amount of a compound selected from the group consisting of
- progesterone,
 - a hydrophobic amine,
 - sphingosine, and
 - a compound of the formula



(I)

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wherein X is O or S;

R_1 is $-C(O)(C_1-C_6)\text{alkyl}$ or $-(CH_2)_n-O-(C_1-C_6)\text{alkyl}$, or $-(CH_2)_n-NR_7R_8$ where n is 0-3, and where each of R_7 and R_8 are independently selected from H and $(C_1-C_6)\text{alkyl}$,

R₂ is H or (C₁-C₆)alkyl;

R₃ is H or (C₁-C₆)alkyl;

R₄ is -C(O)(C₁-C₆)alkyl;

R₅ is H or -(C₁-C₆)alkyl; or R₄ and R₅ together are =O; and

5 R₆ is H or -(C₁-C₆)alkyl or -(CH₂)_n-NR₉R₁₀ where each of R₉ and R₁₀ are independently selected from H and (C₁-C₆)alkyl.

61. The method of claim 60, wherein the compound is progesterone.

62. The method of claim 60, wherein the compound is a hydrophobic amine.

10 63. The method of claim 62, wherein the hydrophobic amine is selected from the group consisting of a phenothiazine, and a tricyclic antidepressant.

64. The method of claim 63, wherein the compound is a phenothiazine.

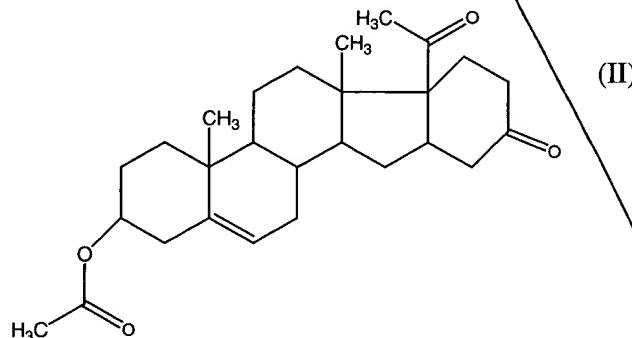
15 65. The method of claim 64, wherein the phenothiazine is selected from the group consisting of trifluoperazine, chlorpromazine, prochlorperazine, triflupromazine, promazine, thioridazine, mesoridazine, piperacetazine, perphenazine, fluphenazine, acetophenazine, and thiethylperazine.

66. The method of claim 63, wherein the compound is a tricyclic antidepressant.

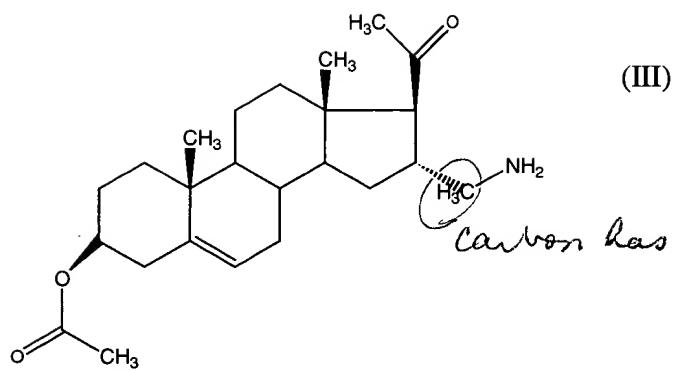
67. The method of claim 66, wherein the tricyclic antidepressant is selected from the group consisting of imipramine, nortriptyline, protriptyline, trimipramine, and doxepin.

68. The method of claim 60, wherein the compound is sphingosine.

20 69. The method of claim 60, wherein the compound is selected from the group consisting of:

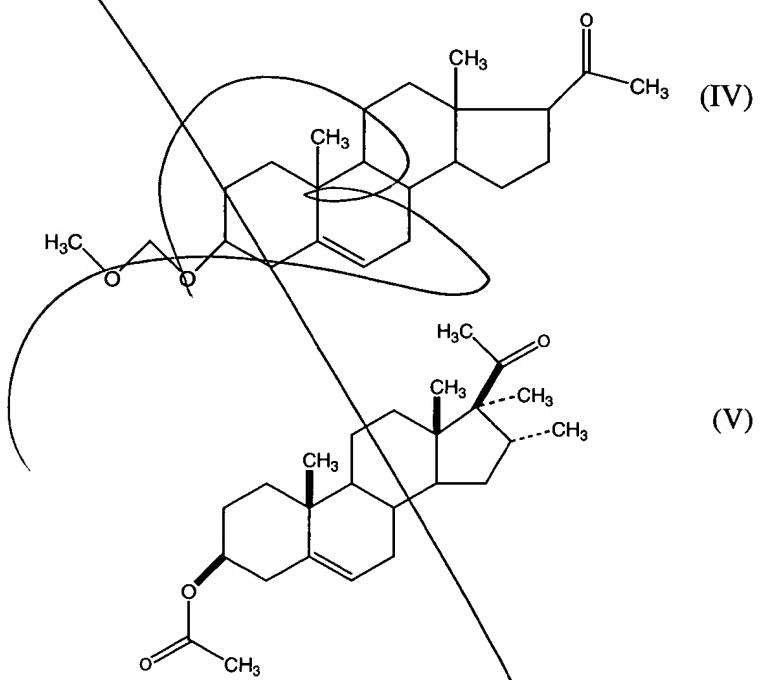


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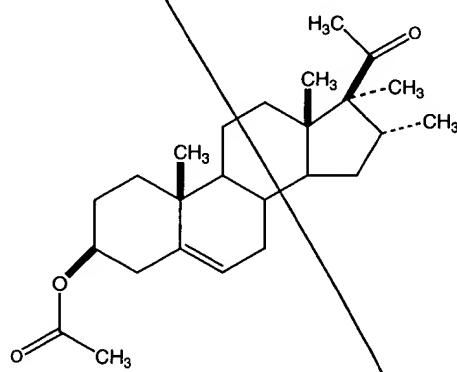


Carbon has valence 75

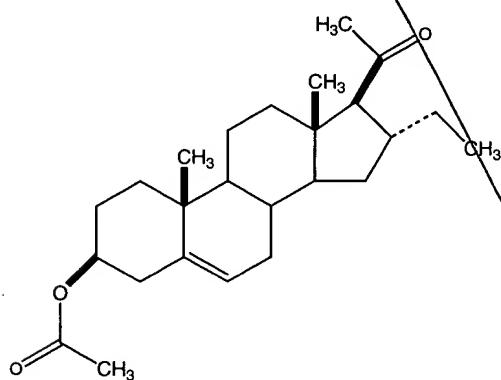
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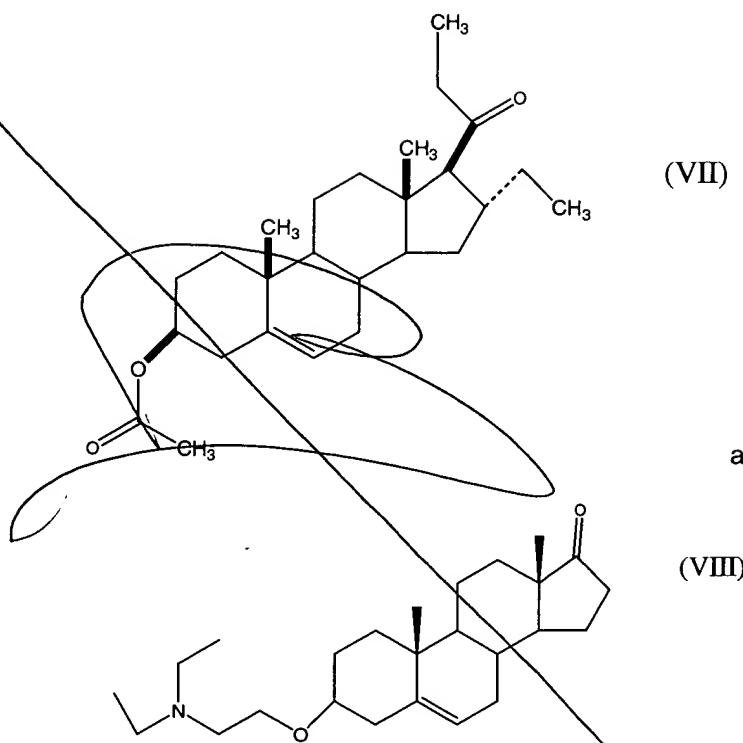


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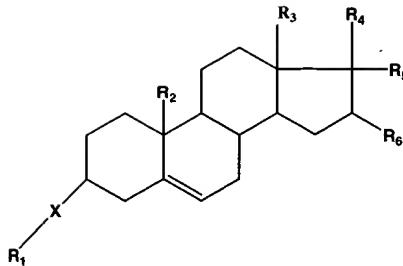


(VI)





- 5 70. A pharmaceutical composition for reducing skin pigmentation, comprising a skin pigmentation reducing effective amount of a compound that effects an alteration in late endosomal/lysosomal trafficking in a skin cell and a pharmaceutically acceptable carrier.
- 10 71. The composition of claim 70, wherein the alteration in late endosomal/lysosomal trafficking is effected by contacting the melanocyte with a compound that is an antagonist of late endosomal/lysosomal trafficking.
72. The composition of claim 70, wherein the alteration in late endosomal/lysosomal trafficking is an alteration in late endosomal/lysosomal cholesterol trafficking.
73. The composition of claim 70, wherein the compound that effects the alteration in late endosomal/lysosomal trafficking is selected from the group consisting of
- 15 (a) progesterone, 514117
 (b) a hydrophobic amine, 5141669
 (c) sphingosine, and
- selected → (d) a compound of the formula



(I)
514/177,482 etc
includes progestrone

wherein X is O or S;

R₁ is $-\text{C}(\text{O})(\text{C}_1\text{-C}_6)\text{alkyl}$ or $-(\text{CH}_2)_n\text{-O-}(\text{C}_1\text{-C}_6)\text{alkyl}$, or $-(\text{CH}_2)_n\text{-NR}_7\text{R}_8$ where n is 0-3, and where each of R₇ and R₈ are independently selected from H and (C₁-C₆)alkyl;

5 R₂ is H or (C₁-C₆)alkyl;

R₃ is H or (C₁-C₆)alkyl;

R₄ is $-\text{C}(\text{O})(\text{C}_1\text{-C}_6)\text{alkyl}$;

R₅ is H or $-(\text{C}_1\text{-C}_6)\text{alkyl}$; or R₄ and R₅ together are =O; and

10 R₆ is H or $-(\text{C}_1\text{-C}_6)\text{alkyl}$ or $-(\text{CH}_2)_n\text{-NR}_9\text{R}_{10}$ where each of R₉ and R₁₀ are independently selected from H and (C₁-C₆)alkyl.

74. The composition of claim 73, wherein the compound is progesterone.

75. The composition of claim 73, wherein the compound is a hydrophobic amine.

76. The composition of claim 73, wherein the hydrophobic amine is selected from the group consisting of a phenothiazine, and a tricyclic antidepressant.

15 77. The composition of claim 76, wherein the compound is a phenothiazine.

78. The composition of claim 77, wherein the phenothiazine is selected from the group consisting of trifluoperazine, chlorpromazine, prochlorperazine, triflupromazine, promazine, thioridazine, mesoridazine, piperacetazine, perphenazine, fluphenazine, acetophenazine, and thiethylperazine.

20 79. The composition of claim 76, wherein the compound is a tricyclic antidepressant.

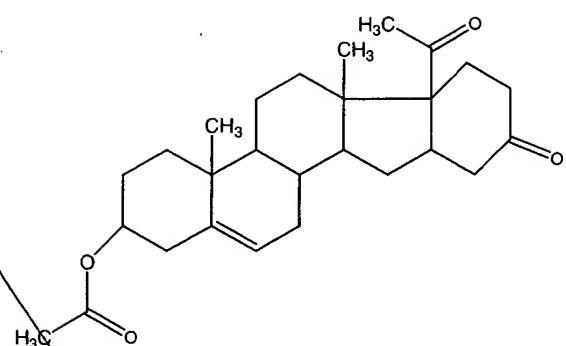
80. The composition of claim 79, wherein the tricyclic antidepressant is selected from the group consisting of imipramine, nortriptyline, protriptyline, trimipramine, and doxepin.

81. The composition of claim 73, wherein the compound is sphingosine.

82. The composition of claim 73, wherein the compound is selected from the group 25 consisting of:

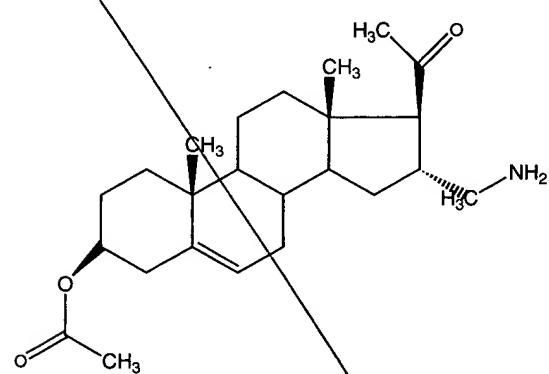
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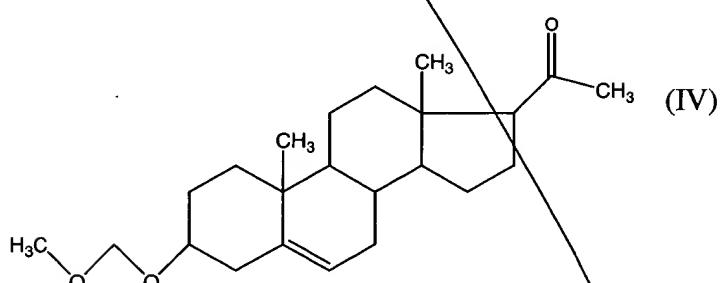


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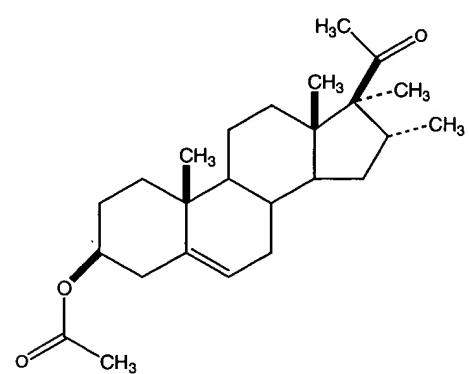
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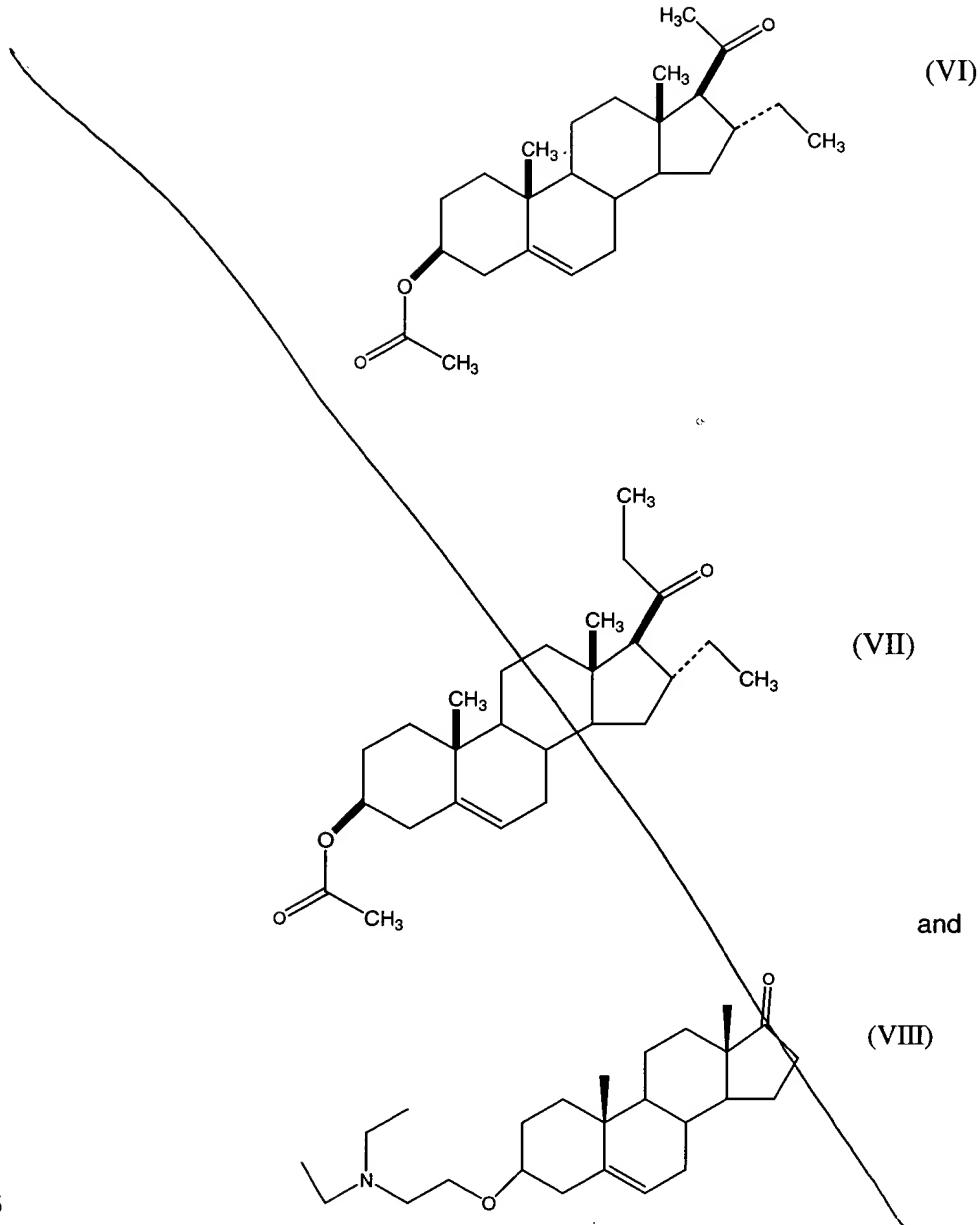
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83. A method of activating melanogenesis comprising contacting a melanocyte with diminished or absent P protein activity with a pharmaceutically effective amount of a compound that inhibits ATPase, whereby the inhibition of ATPase results in an activation of melanogenesis in the melanocyte with diminished or absent P protein activity.

10 84. The method according to claim 83, wherein the melanocyte with diminished or absent P protein activity is contacted with a pharmaceutically effective amount of a compound selected from the group consisting of baflomycin, concanamycin, and derivatives thereof.



85. The method according to claim 83, wherein the melanocyte with diminished or absent P protein activity is contacted with a pharmaceutically effective amount of baflomycin or a derivative thereof.
86. The method according to claim 83, wherein the melanocyte with diminished or absent P protein activity is contacted with a pharmaceutically effective amount of concanamycin or a derivative thereof.
87. A method of treating tyrosinase-positive, oculocutaneous albinism in an individual in need thereof, the method comprising contacting skin of the individual with a pharmaceutically effective amount of a compound that inhibits ATPase, whereby the inhibition of ATPase results in an activation of melanogenesis in the individual.
88. The method according to claim 87, wherein the skin of the subject is contacted with a pharmaceutically effective amount of a compound selected from the group consisting of baflomycin or a derivative thereof and concanamycin or a derivative thereof.
89. The method according to claim 88, wherein the skin of the subject is contacted with a pharmaceutically effective amount of baflomycin or a derivative thereof.
90. The method according to claim 88, wherein the skin of the subject is contacted with a pharmaceutically effective amount of concanamycin or a derivative thereof.
91. A kit comprising a pharmaceutically effective amount of a compound that modulates melanogenesis by affecting P-protein-function, inhibiting late endosomal/lysosomal trafficking, or inhibiting an ATPase.
92. The kit of claim 91, further comprising a set of written instructions describing how to use the compound to modulate skin pigmentation.

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